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A Comparison of Skull and Femur Lead Levels in Adult Rats

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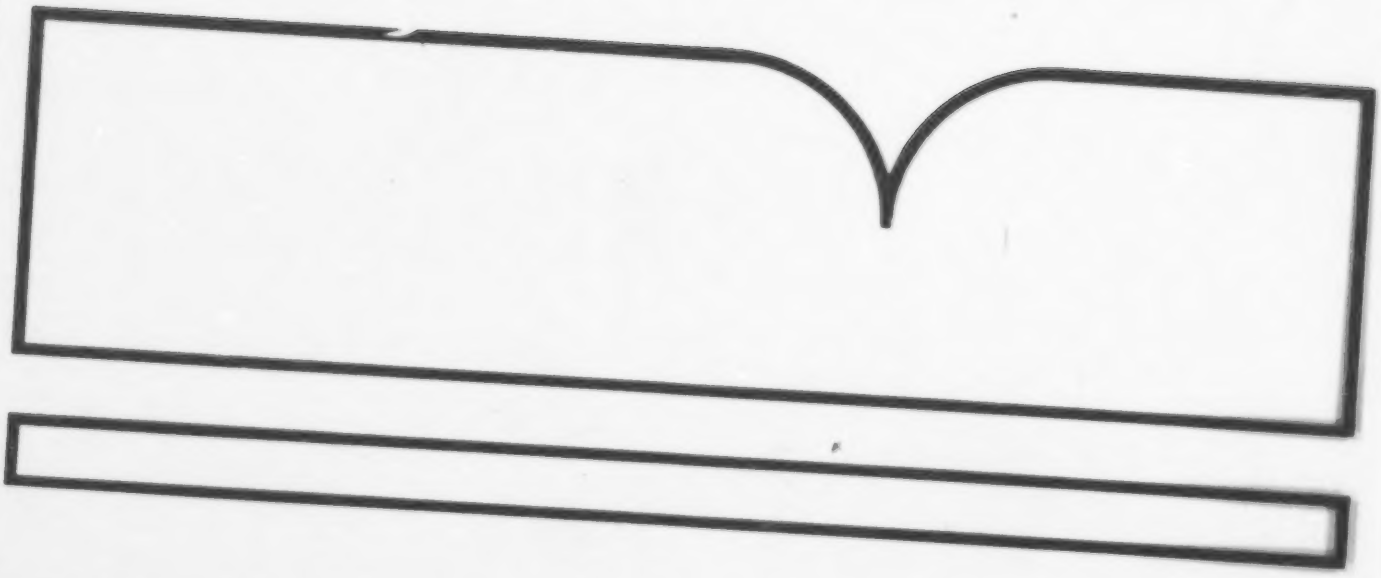

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16. ABSTRACT

The purpose of the study was to elucidate the relationship between skull and femur lead levels in laboratory rats. Forty-eight female rats were given one of four lead chloride drinking water solutions: 0.05, 0.58, 17, or 352 ppm lead. Two animals from each group were sacrificed after 3, 6, 9, 12, 15, and 24 weeks of treatment. Both femurs and the frontal and parietal bones of the skull were removed from each animal and analyzed for lead concentration by atomic absorption spectroscopy. A significant accumulation of lead was observed in femurs and skull bones only from animals in the 352 ppm lead treatment group. The lead concentrations of the femurs were significantly higher than skull lead concentrations for all groups and this relationship was described using a linear regression equation.

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A Comparison of Skull and Femur Lead Levels in Adult Rats

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The purpose of the study was to elucidate the relationship between skull and femur lead levels in laboratory rats. Forty-eight female rats were given one of four lead chloride drinking water solutions: 0.05, 0.58, 17, or 352 ppm lead. Two animals from each group were sacrificed after 3, 6, 9, 12, 15, and 24 weeks of treatment. Both femurs and the frontal and parietal bones of the skull were removed from each animal and analyzed for lead concentration by atomic absorption spectroscopy. A significant accumulation of lead was observed in femurs and skull bones only from animals in the 352 ppm lead treatment group. The lead concentrations of the femurs were significantly higher than skull lead concentrations for all groups and this relationship was described using a linear regression equation.

INTRODUCTION

Bone tissue is the major site of lead accumulation in animals. Of the total amount of lead in the human body, 90 to 95% is found in a nondiffusible form in the skeleton (Horiuchi *et al.*, 1959; Schroeder and Tipton, 1968; Knelson *et al.*, 1973). Measurements of bone lead concentrations in laboratory animals under experimental conditions provide information on the relationship between lead accumulation and its health effects. In addition, evaluation of the environmental accumulation of lead usually involves measurement of bone lead concentrations in wild rodents. For these types of studies, the lead concentrations of long bones, such as the femur and tibia, are used as an index of skeletal lead levels (Welch and Dick, 1975; Getz *et al.*, 1977).

Long bones are generally analyzed for lead content because they are easily dissected from surrounding tissues. However, they are not necessarily the most appropriate bones for an estimate of total skeleton lead levels. Examination of the concentration of lead (^{210}Pb) in the skull, mandible, rib, and tibia of humans by Holtzman (1963) provided evidence that lead levels in the flat bones of the skull were most representative of lead concentrations in the total skeleton. In his study, the concentration of lead in the skull deviated 16% from the mean value which was the smallest deviation of all bones examined. Lead concentration of the rib deviated highest from the mean value and represented the upper limits of lead in the body. He further reported that lead concentrations of the tibia and mandible were below the mean value.

Since skeletal lead estimates in small mammals are generally based on femur or tibia lead concentrations, information on skull lead concentrations could provide a more accurate estimate of skeletal lead levels. This study was conducted to determine the relationship between skull and femur lead distribution in laboratory rats and examine whether it is similar to that in humans.

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METHODS

Forty-eight female Wistar-strain laboratory rats (245–316 g) were individually confined to 20 × 51-mm raised cages with food (Wayne Lab-Blox) and lead drinking water solutions continuously available. Room temperature was maintained at 22°C (72°F), relative humidity at 38%, and the animals were kept on a 10½ hr on–13½ hr off light cycle. Standard laboratory animal care practices were observed in accordance with the Regulations of the American Physiological Society for the Care and Use of Animals.

The rats were separated into four treatment groups of 12 animals each. Each group received one of four drinking water solutions of either 0.05, 0.58, 17, or 352 ppm lead as lead chloride in deionized–distilled water. The solutions were analyzed weekly for lead concentration levels using atomic absorption spectroscopy methods. After 3, 6, 9, 12, 15, and 24 weeks of lead treatment, two animals from each group were sacrificed using nitrogen. The parietal and frontal bones of the skull and both femurs were removed from each animal and scraped clean of surrounding tissue. Bone marrow was removed and discarded.

Methods for preparation of bones and food samples for lead analysis were adopted from previously described methods (Gorsuch, 1970; Abu-Samra *et al.*, 1975). Bone samples were oven-dried for 24 hr at 27°C and then placed overnight in 15 to 20 ml redistilled concentrated nitric acid (G. Frederick Smith Chemical Co.). The samples were digested with nitric acid in a Litton Minutemaster microwave oven adopted for this purpose. Three samples of food were also digested for lead analysis and all digested samples were frozen at −20°C in acid-washed polyethylene vials until analysis.

Bone, food, and drinking water samples were analyzed for lead using Perkin–Elmer 403 and 603 atomic absorption spectrometers equipped with air:acetylene flames. A standard calibration procedure, with periodic analysis of blanks and spiked samples, was followed. All samples were analyzed in duplicate runs and a randomly selected spiked sample was analyzed between every ten samples. The instrument was calibrated with a blank and standard, then calibrated after every 40 samples.

The data were corrected to dry weight values for the skull and femur samples after atomic absorption analysis. The data were statistically analyzed using a three-factor analysis of variance test with treatment levels, weeks of treatment, and skull and femur lead concentrations as factors. All data were considered significant at the $P < 0.05$ level. A linear regression analysis was used to describe the relationship between femur and skull lead concentrations. An r value greater than 0.8 was considered highly significant.

RESULTS

Calculated Lead Intake from Treatment Solutions and Food

Table 1 lists the amount of lead ingested from treatment solutions between the 3rd and 24th weeks of the study. Kostial *et al.* (1971) reported the absorption of lead from the gastrointestinal tract of 3-month-old rats to be 16%. Using this percentage, we calculated that for the 0.05 and 0.58 ppm lead treatment groups,

TABLE 1
LEAD INTAKE IN RATS DOSED WITH LEAD CHLORIDE

Weeks of treatment	Treatment (μg/ml lead)			
	0.05	0.58	17	352
3	0.0[a]	0.4	13.1	207
6	0.1	0.9	27.3	473
9	0.1	1.1	31.8	761
12	0.2	1.6	47.5	951
15	0.2	2.1	55.3	1251
24	0.4	3.6	99.3	2122

[a] Data are expressed as mg lead.

less than 1 mg of lead was absorbed from the drinking water solutions. The animals in the 17 ppm lead group absorbed 2 to 16 mg of lead from ingestion of the solutions between 3 and 24 weeks while, during the same period, the 352 ppm lead treatment group absorbed 33 to 340 mg of lead.

The food was analyzed for lead concentration and found to contain 5 μg/g lead. Christensen *et al.* (1975) reported that the food intake of adult female rats was 10 g per day. Based on this figure, the lead intake from the food was calculated as 50 μg/day throughout the study, and the total lead intake from food calculated at 1.0 to 8.4 mg for 3 to 24 weeks of treatment. With a 16% absorption rate, this was 0.2 to 1.3 mg lead absorbed from food for 3 to 24 weeks, respectively. Lead intake from food was greater than intake from water in the two lowest groups; food lead intake was 9% of the total lead intake for the 17 ppm lead treatment group, and less than 1% for the 352 ppm lead treatment group.

Lead Concentrations of Femur and Skull Samples

Figure 1 illustrates the lead concentrations of femur samples, dry weight, from animals sacrificed during the 24-week period. The highest average femur lead concentration was 116.8 μg/g in the 352 ppm lead treatment group after 24 weeks of lead treatment; the lowest was 28.2 μg/g lead after 12 weeks of 0.58 ppm lead treatment. Except for the ninth week at 0.05 ppm lead, the highest lead concentrations were found in animals which received 352 ppm lead. In Fig. 1, the large standard deviation at the ninth week for the 0.05 ppm treatment group was due to a large difference in femur lead concentrations between the two animals in that group. One animal had a femur lead concentration of 28.3 μg/g while the other animal had a concentration of 102.9 μg/g lead. Similarly, the large standard deviation for the 352 ppm lead treatment group at the ninth week resulted from a femur lead concentration of 39.9 μg/g in one animal and 120.0 μg/g in the other animal. We suspect that the largest concentrations in both treatment groups were outlying values. However, since there were only two animals per group, we could not test for this and the data were included in Fig. 1.

Figure 2 illustrates lead concentrations of skull samples during the 24-week period. Standard deviations for the skull data were high for the 0.05 and 352 ppm lead treatment groups at the ninth week, similar to that observed for these animals

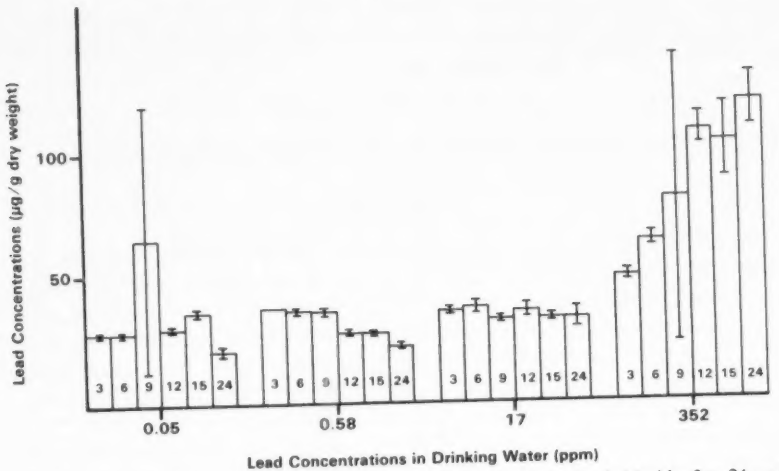

FIG. 1. Lead concentrations, dry weight, of femurs from rats ingesting lead chloride, 3 to 24 weeks. Data are expressed as $\bar{X} \pm$ SD for two animals per treatment group per week. The femurs for only one animal were analyzed for the third week in the 0.58 ppm lead treatment group.

in the femur lead concentration data. The individual skull lead concentrations for the 0.05 ppm group were 25.8 and 51.6 μg/g lead and for the 352 ppm group 24.5 and 61.7 μg/g lead. Outlying values were again suspected but since there were only two animals per group, a test for this could not be performed and all data were included in Fig. 2.

Al three-factor analysis of variance of femur and skull lead concentrations showed that a significant accumulation of lead in both femur and skull samples occurred only in those animals in the 352 ppm lead treatment group ($P < 0.05$). The femur lead concentrations were significantly higher than the skull lead con-

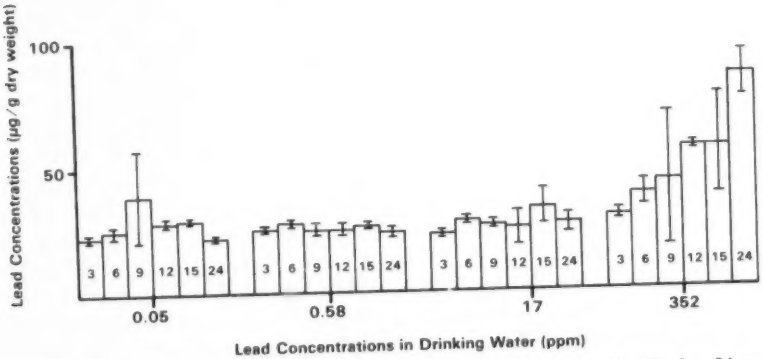

FIG. 2. Lead concentrations, dry weight, of skulls from rats ingesting lead chloride, 3 to 24 weeks. Data are expressed as $\bar{X} \pm$ SD for two animals per treatment group per week.

centrations for all groups ($P < 0.05$) and a linear regression analysis was used to describe the relationship between femur and skull lead levels. The linear equation which describes their relationship was:

$$S = 1.79X - 12.07,$$

where S = skull lead concentrations and X = femur lead concentration ($r = 0.93$).

DISCUSSION

The purpose of the study was to evaluate the relationship between skull and femur lead concentrations in laboratory rats given lead in their drinking water. Long bones such as femur and tibia are frequent indices of skeletal lead levels in small mammals in field and laboratory studies. However, Holtzman (1963) reported lead levels in flat bones of the human skull were most representative of total skeletal lead concentrations. Since this might also be true for small mammals, this study was conducted to provide prediction of skull lead levels from routinely measured lead concentrations in long bones (femur or tibia). We also determined what doses of lead must be administered to laboratory rats to detect a significant accumulation of lead in bone.

We observed that femur lead concentrations, dry weight, for rats which ingested 0.05–17 ppm lead were 28.2–40.4 μg/g. These data agreed with Getz *et al.* (1977) who reported femur lead levels of small mice (*Mus musculus*) as 9.3 ± 9.0 to 23.5 ± 46.8 μg/g ($\bar{X}$ ± SD). Similarly, our data were well within the range of 45.5 to 195.0 μg/g lead of femur and humerus bones in small mammals reported by Sharma and Shupe (1977).

The amount of lead accumulated in the skeleton seemed to vary in different bones. We observed that in rats, lead concentrations in the skull were significantly lower than femur irrespective of the amount of lead ingested. However, Barry and Mossman (1970) observed that the dense petrous temporal bone of the skull contained the highest lead concentration of bones analyzed. The tibia contained the second highest concentration. Also, they reported that the steepest rise in lead concentration with age occurred in the temporal bone. Similarly, Khandekar and Anand (1971) reported that the average concentration of ^{210}Pb in long bones was less than the average concentration in skull and vertebrae. Rib contained the highest concentration of lead in human bones and was as much as 38% higher than the mean concentration (Holtzman, 1963; Khandekar and Anand, 1971). Although Holtzman (1963) reported skull lead levels were higher than femur, our observations of an opposite relationship agree with Smith *et al.* (1970). These authors observed that femur lead was higher than lead in the skulls of rabbits.

Differences in lead concentrations within bones and between species could be due to structural differences in different types of bones. Femur and tibia, or long bones, are composed of trabecular bone tissue covered by a variable depth of compact bone. Skull and other flat bones are composed primarily of dense compact bone tissue on the external surface which extends into the interior as laminae. The greater femur lead concentration when compared with that of the skull in rats, which we observed, was opposite to that of human bone lead. This could be due to a greater amount of compact bone in rat femurs than skulls. On the other hand,

different areas of the skull could contain different concentrations of lead. Holtzman (1963) examined the entire skull, whereas we analyzed only parietal and frontal bones. In fact, Barry and Mossman (1970) found that the temporal bone contained the highest lead concentration of all bones examined.

The relationship between femur and skull lead levels in rats was described by a linear regression equation. This had not been previously reported and should facilitate prediction of skull lead levels in studies in which only femur concentrations are measured. The ability to predict lead in the skull could be particularly useful in evaluation of total-body lead burden in both laboratory and field studies which monitor accumulation and distribution of lead in the environment.

No significant differences were observed in lead concentrations of femurs from animals in the 0.05, 0.58, and 17 ppm lead groups. In fact, the amount of lead calculated as ingested from food alone was larger than the intake from lead drinking water solutions in the 0.05 and 0.58 ppm groups. A significant accumulation of lead in femur and skull samples was observed during the study only in animals which received the 352 ppm lead treatment. We concluded that for future laboratory studies, oral doses greater than 17 ppm must be administered to observe a significant accumulation of lead in the skeleton.

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